ISOLATION OF ARISTOLOCHIC ACID FROM *ARISTOLOCHIA CLEMATITIS* NATIVE IN HUNGARY. THE PREPARATION OF PHYSIOLOGICALLY ACTIVE ARISTOLOCHIA DERIVATIVES

By GY. SCHNEIDER

Institute of Organic Chemistry, The University, Szeged

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A material of strong physiological activity with the composition of $C_{17}H_{11}O_7N$ has been isolated from *Aristolochia clematitis* native in Hungary. The same compound had been found in other species of *Aristolochia*. An interesting property of the compound is that its nitrogen function is contained in a nitro group. Several derivatives have been prepared for pharmacological investigation.

Species of *Aristolochia* are native mostly in countries around the Mediterranean, however, a number of them is to be found also in other parts of Europe. The active principle of the plant is a stimulative poison, and several experiments have been made to find a use for it in the medical practice. Some species of *Aristolochia* have been used as popular drugs to produce abortion. The chief active principle in the *Aristolochia* species is aristolochic acid, a yellow and extremely bitter material, having an acidic character. In 1892 POHL [1] isolated a yellow crystalline solid from the seeds of *Aristolochia rotunda*, and from the result of combustion analysis he established the empirical formula $C_{32}H_{22}O_{13}N_2$. The material showed acidic properties, and it gave a well defined barium salt. POHL called the active principle obtained by him aristolochine, and he was the first to make pharmacological experiments with it. In 1895 HESSE [2] isolated three acidic substances from *Aristolochia argentina*, namely aristinic acid ($C_{18}H_{13}O_7N$), aristidinic acid (which is considered by him as an isomer of aristinic acid), and aristolic acid ($C_{15}H_{11}O_7N$); all the three materials showed rather similar properties. A yellow crystalline and very bitter solid was isolated in 1922 by CASTILLE [3] from *Aristolochia sipho l'Hérit;* its properties were similar to those of the material obtained by POHL, however, it had the empirical formula $C_{17}H_{11}O_7N$. The compound gave off ammonia when fused with potassium hydroxide. The residue showed the characteristics of antrachinone, from which it was concluded that the material had an anthracene sceletal structure, probably with a tertiary nitrogen atom on it. In 1943 ROSENMUND and REICHSTEIN [4] investigated the material isolated from *Aristolochia sipho.* An active principle of the composition $C_{17}H_{11}O_7N$ could be isolated by extraction, which was identical with the ma-

terial obtained by CASTILLE. The compound could be sublimed under reduced pressure, however, not without decomposition. Treatment with diazomethane gave a derivative with the empirical formula $C_{18}H_{13}O_7N$ which was found to be very stable againts hydrolysis. The parent compound as well as its derivatives were optically inactive. Hydrogenation in the presence of platinum oxide resulted in the formation of a light yellow material, which was shown by analysis to have the composition $C_{18}H_{13}O_7N \cdot 0{,}5\,H_2O$. Decarboxylation with copper in quinoline afforded $C_{16}H_4O_5N$, and the reaction confirmed the assumption that aristolochic acid was a monobasic acid, and the acidic character was not derived from the presence of phenolic hydroxyl, as it was thought formerly. However, this investigation did not elucidate the character of the nitrogen atom and of the other functional groups of the molecule. PAILER *et all.* [5] started a research in 1956, and found that aristolochic acid isolated from *Aristolochia clematitis* L. was identical with the material of Rosenmund and Reichstein. They pointed out that the various acids of *Aristolochia* as described by different authors were not uniform preparations, and this fact rendered the investigations very difficult. The aristolochic acid isolated by them showed the empirical formula $C_{17}H_{11}O_7N$ which had been considered most probable also previously. The usual method of methoxyl determination gave but very little CH_3J, which had led earlier researchers to erroneous conclusions concerning the functional groups of the molecule. When the method was developed to be come more punctual, the presence of one methoxyl group was demonstrated. Catalytic hydrogenation of aristolochic acid permitted the isolation of a material having neutral character. Hydrogenation of the decarboxylated acid gave rise to a basic material from which a hydrochloride, picrate and acetate could be prepared. Diazotation followed by hydrolysis gave a red material. These different experimental results suggested the presence of a nitro group in aristolochic acid which could be reduced to an amino group, when the amine obtained will form a lactam. This assumption has been substantiated also by the evidence of infrared spectra. The presence of the methylenedioxy group was demonstrated by the method of PAVOLINI [6]. Distillation of the compound with zinc dust gave phenanthrene. After having established the nature of the functional groups, PAILER *et al.* found out their relative positions by oxidative degradation. This investigation gave the structure of aristolochic acid as 3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene-1-carboxylic acid. The formula was proved by synthesizing 3,4-methylenedioxy-10-acetamidophenanthrene, which was also obtained from the degradation of aristolochic acid. The synthesis was carried out by condensing homopiperonylic acid with o-nitrobenzaldehyde, followed by PSCHORR [7] ring closure.

We have found that the active principle of the *Aristolochia clematitis* native in Hungary was in all respects identical with the material isolated by ROSENMUND and REICHSTEIN and it had the formula as given by PAILER. It is interesting that the nitrogen atom of the molecule is contained in a nitro group, which fact suggested further pharmacological investigation. There are only very few nitrogen containing compounds occurring in nature which possess nitro group. Such rare compounds are chloramphenicol, isolated in 1947, and β-nitropropionic acid found not much later. A detailed pharmaco-

logical study of aristolochic acid has been carried out recently by MÉHES *et al.* [8, 9].

The chemical investigation of aristolochic acid presented two problems. First, a method was to be developed for the extraction of aristolochic acid from *Aristolochia clematitis* native in Hungary; second, the isolated acid had to be modified in various ways. To prepare derivatives for pharmacological study, first of all we wanted to eliminate the action of the acidic carboxyl group of the molecule. This was partly done by decarboxylation, partly by preparing various esters of the acid. Esterification could not be carried out in the conventional way, thus the acid chloride was prepared first, which gave the desired ester on alcoholysis. Ethyl, n-propyl and n-butyl esters have been prepared in this way. The methyl ester was obtained by means of diazomethane. (Fig. 1.)

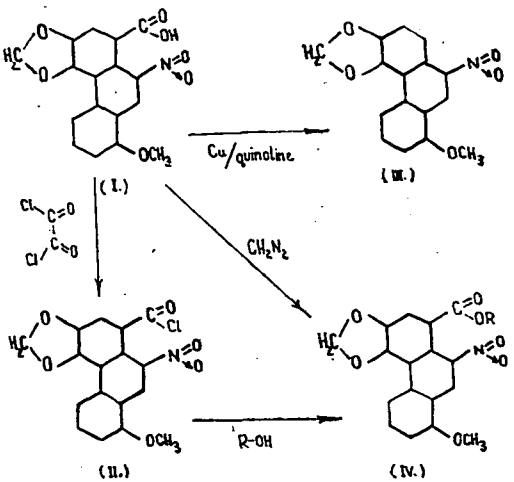

Fig. 1

Experimental

Isolation and purification of aristolochic acid (I)

Roots of *Aristolochia clematitis*, collected during the autumn season, were cleansed and dried under reduced pressure at 60° C. After drying, the material was ground in a beating mill. The pulverized substance was extracted with cold petroleum ether to dissolve fatty components. The material was then extracted with ethanol, until the solvent ceased to show a brown colour. The extraction was finished by employing ethanol containing 1 % sodium ethoxide. The ethanol and sodium ethoxide extracts were combined and evaporated at 50° C under reduced pressure. The residue was a mobile, brown, oily liquid which was dissolved in 1 % sodium hydroxide, and acidified with 1 % sulphuric acid. A brownish-yellow precipitate was obtained which was centrifuged, washed free of sulphate ions and dried at room temperature over phosphorus pentoxide to give a brown powder which had an extremely bitter taste. The crude aristolochic acid obtained this way was extracted with petroleum ether in a Soxhlet apparatus. The extraction was then continued using diethylether. After having performed the operation for several days, aristolochic acid separated as a yellow solid in the extraction flask. The crust of crystals was dissolved in dioxane, treated with decolourizing carbon, and crystallized by the addition of a small amount of water. Orange-yellow plates

were obtained. Aristolochic acids crystallized from dioxane gave no exactly reproducible analyses, because varying amounts of dioxane may be bound by the acid. Material for analysis was crystallized from butyl alcohol. M. p. 283—285° C (*decomposition*). (*The determination of the melting point* was carried out according to the microanalytical method of A. KOFFLER.) Analysis: $C_{17}H_{11}O_7N$ requires C 59,82; H 3,25; N 4,11; OCH_3 9,09%. Found C 59,94; H 3,24; N 4,17; OCH_3 9,12%.

Sodium salt of aristolochic acid

Aristolochic acid (200 mg) was dissolved in anhydrous ethanol containing a calculated amount of sodium ethylate, and the solution was evaporated to dryness. The residue was dissolved in butyl alcohol. The sodium salt crystallized from this solution in long, bright red needles, m. p. 275—278° C (decompn.). Analysis: $C_{17}H_{10}O_7N$ Na requires C 56,19; H 2,77; N 3,85 %. Found C 56,27; H 2,68; N 3,89%.

Decarboxylated aristolochic acid (III)

Aristolochic acid (80 mg) was dissolved in 10 ml of quinoline, 50 mg of copper powder was added, and the material refluxed for 15 minutes. After cooling it was diluted with chloroform, filtered from copper, washed with diluted hydrochloric acid, then with $NaHCO_3$ solution and water. The chloroform solution was dried, and the chloroform distilled. The residue was recrystallized from a mixture of chloroform and methanol, to give long yellow needles, m. p. 212° C. Found: C 64,74; H 3,78; N 4,85%. Calculated for $C_{16}H_{11}O_5N$: C 64,65; H 3,73; N 4,71%.

Chloride of aristolochic acid (II)

Aristolochic acid (500 mg) was dissolved in 50 ml of anhydrous dioxane and a great excess of oxalyl chloride (8 ml) was added. The solution was allowed to stand at room temperature for one day, and it was evaporated under reduced pressure. A greenish-yellow crystalline material was obtained which was recrystallized from chloroform. M. p. 275—278° C (decompn.) Found: C 56,80; H 2,89; N 3,72; Cl 9,68%. Calculated for $C_{17}H_{11}O_6N$ Cl: C 56,76; H 2,80; N 3,89; Cl 9,63%.

Methyl ester of aristolochic acid (IV)

Aristolochic acid (100 mg) was dissolved in 20 ml of anhydrous dioxane and an excess of diazomethane dissolved in ether (20 ml) was added. A crystalline precipitate was obtained in an hour. After evaporating the ether, the material was dissolved in chloroform, and the solution was washed with $NaHCO_3$, dried, and evaporated to dryness. Recrystallization from methanol gave thin needles, m. p. 280—283° C. Found: C 60,72; H 3,75; N 3,97; OCH_3 17,60%. Calculated for $C_{18}H_{13}O_7N$: C 60,85; H 3,68; N 3,94; OCH_3 17,46%.

Lower homologous esters of aristolochic acid (V)

The chloride of aristolochic acid (50 mg) was dissolved in 10 ml of chloroform, and a large excess of the esterifying alcohol added. The mixture was refluxed for one hour. After cooling, the solution was evaporated, the residue dissolved in chloroform, washed with $NaHCO_3$ solution, dried, and concentrated to small volume which resulted in the crystallization of the ester.

M. p. of ethyl ester: 282—283° C (decompn.). Found: C 61,92; H 4,15; N 3,86%. Calculated for $C_{19}H_{15}O_7N$: C 61,79; H 4,09; N 3,79%.

M. p. of n-propyl ester 286—288° C (decompn.). Found: C 62,59; H 4,54; N 3,76%. Calculated for $C_{20}H_{17}O_7N$: C 62,67; H 4,47; N 3,65%.

M. p. of *n*-butyl ester 286—290° C (decompn.). Found: C 63,55; H 4,93; N 3,58%. Calculated for $C_{21}H_{19}O_7N$: C 63,47; H 4,81; N 3,52%.

References

[1] *Pohl, J.:* Arch. exper. Pathol, Pharmakol. **29** 282 (1892).
[2] *Hesse, O.:* Arch. Pharmaz. **233**, 684 (1895).
[3] *Castille, A.:* J. Pharmacie Belgique **4**, 125, 141, 569 (1922).
[4] *Rosenmund, H., T. Reichstein:* Pharmaceut. Acta Helv. **18**, 243 (1943).
[5] *Pailer, M., L. Belochlav, E. Simonitsch:* Mh. Chem. **87**, 249 (1956).
[6] *Pavolini, A., A. Malatesta:* Ann. Chim. applic. **37**, 495 (1947).
[7] *Pschorr, R., H. Busch:* Ber. dtsch. chem. Ges. **40**, 2001 (1907).
[8] *Méhes, J., L. Decsi, F. Varga, S. Kovács:* Arch. exper. Pathol. u. Phar. **234**, 548 (1958).
[9] *Schneider, Gy., J. Szöke, S. Kovács:* Arch. exper. Pathol.. u. Phar. **234**, 566 (1958).

ИЗОЛИРОВАНИЕ АРИСТОЛОКИА-КИСЛОТЫ ИЗ ARISTOLOCHIA CLEMATITIS РАСТУЩЕЙ В ВЕНГРИИ. ИЗГОТОВЛЕНИЕ АРИСТОЛОКИА-ПРОИЗВОДНЫХ, ЕФФЕКТИВНЫХ ФАРМАКОЛОГИЧЕСКИ

Дь. Шнейдер

Из *Aristolochia clematitis* растущей в Венгрии было изолировано вещество, соответствующее составу $C_{17}H_{11}O_7N$ которое обладает сильным фармакологическим действием. Такое же вещество содержится и в других породах Aristolochia. Соединение представляет собой интерес, потому что функция азота носится в нем нитрогруппой. Для фармакологических исследований были изготовлены некоторые из его производных.